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BIOCONVERSION OF CHEESE WHEY TO BIOMASS AND BIOPROTEIN BY YEAST CULTURES

Hasani Ahmadreza[1,2]*; S.I. Aliyeva[2] and S.A. Mirhadi[3]; N. Vaseji[3] and Abdolhoseini

1- *Animal Science Research Institute, Tabriz-Iran*

2- *Department of Microbiology, Baku State University,Baku-Azerbaijan Republic*

3- *Animal Science Research Institute, Karaj-Iran*

***Corresponding** E-mail: drhasani124@yahoo.com*

ABSTRACT

This research emphasizes on bioconversion of cheese whey by using of six yeast strains culture. Cheese whey, in Iran and Azerbaijan republic, is treated as a waste and discharged in nature approximately without any treatment which creates pollution problems. It is a by-product of the dairy industry and contains usually high levels of lactose (4-6%), low levels of nitrogenous compounds and small amounts of vitamins and minerals. Lactose, the main nutrient in cheese whey, can be economically utilized by its conversion to biomass and bioprotein. For this purpose, 6 yeast strains: ***Trichosporon pullulans, Candida curvata, Cryptococcus podzolicus, Bullera oryza, Cryptococcus laurentii*** and ***Cryptococcus flavus*** were chosen among isolated yeasts from natural habitats in different forest ecosystems, for their growth and single cell protein content on cheese whey. These yeast strains were tested for their ability to produce SCP from cheese whey. Among these strains, *Cryptococcus laurentii* had the most SCP production from whey with the yield of 18.84 ± 0.50 g/lit. The enrichment of cheese whey by minerals as nitrogen source increased biomass yield. Preliminary tests in our laboratory showed that among the chosen yeasts in this survey, 2 yeast strains (*Cryptococcus laurentii* and *Cryptococcus flavus* respectively) as shown in the text had relatively high biomass production when cultivated on cheese whey. They were further grown on sterilized, non-sterilized and enrichment fresh sweet cheese whey.
Keywords: Bioprotein – cheese whey – lactose – yeast - SCP.

1. INTRODUCTION

Cheese whey, in Iran and Azerbaijan republic, is treated as a waste and discharged in nature without any treatment which creates pollution problems. It is a by-product of the dairy industry and contains usually high levels of lactose (4-6%), low levels of nitrogenous compounds and small amounts of vitamins and minerals (Ben-Hassan et al. ,1994; Carlotti et al,1991; Moeine et al,2004 and Omar,1991).

Lactose, the main nutrient in whey, can be economically utilized by its conversion to single-cell protein (SCP). Dried whey has been widely used as a feed for cattle although its nutritional value is low, because of its low organic nitrogen content. The production of microbial protein from whey reduces the BOD of the effluent by converting as much as 90% of the lactose to protein(Mansour et al, 1993; Moeine et al,2004 and Omar,1991). According to Shah et al. only 56% of the whey solids were utilized for human food and animal feed (Shah et al, 1993).

Many studies of microbial protein production from whey have been reported, mostly using yeasts (Gonzales Siso,1996; Jakobsen,1996; Klaus,1996 and Mawson,1994). In the studies reported so far, certain yeasts have been used for conversion of whey lactose into biomass. Although there are other lactose utilizing microorganisms, these have not been studied for their conversion efficiency of whey lactose. A major problem in using whey as a fermentation medium has been the fact that relatively few organisms are able to ferment lactose (Mansour et al,1993). In order to discover more efficient microorganisms for this purpose in this work 6 yeast stains were chosen among isolated and screened yeasts from natural habitats in different forest ecosystems to select the most suitable organisms for their bioprotein production. They were grown on sterilized (SW), non-sterilized (n-SW) and enrichment sterile (en-SW) fresh sweet cheese whey.

2. MATERIALS AND METHODS

2-1. Microorganisms

Used cultures or yeast strains in this research were isolated from different biogeocenose of Azerbaijan republic forest and grassland structure. All of them were collected and preserved as the yeast culture collection in microbiology department of biology faculty, Baku state university (Hasani et al, 2007).

Table 1. Growth of lactose-metabolizing yeasts on whey.

No.	Strains	No.	Strains
1	*Trichosporon pullulans*	4	*Bullera oryza*
2	*Candida curvata*	5	*Cryptococcus laurentii*
3	*Cryptococcus podzolicus*	6	*Cryptococcus flavus*

2-2. Types of whey used as substrates

The fresh sweet cheese whey was obtained from dairy producing centers in the region of Ismailli in north of Azerbaijan republic. Samples containing whey were collected in sterile 500 mL bottles and brought to the laboratory in a cooler box. The whey was preserved by freezing in 200 ml capacity Erlenmeyer flasks until working or culturing them in order to prevent lactose hydrolysis by Lactobacillus.

C I) Control **I,** Distilled water containing of 4% lactose
C II) Control **II,** Distilled water containing of 2% glucose
S1) Non-sterile whey (**n-SW**)
S2) Sterile whey (**SW**)
S3) Enrichment Sterile whey (**en-SW**) with minerals (g/l whey): $(NH_4)_2SO_4$, 3; Na_2HPO_4, 0.2; $MgSO_4$. $7H_2O$, 0.1; NaCl, 0.1; $CaSO_4$. $2H_2O$, 0.1; $FeSO_4$. $7H_2O$, 0.025; $ZnSO_4$. $7H_2O$, 0.0075; $MnSO_4.4H_2O$, 0.005; $CuSO_4.5H_2O$, 0.001 and, H_3BO_3, 0.0005.

2-3. Media for screening:

The initial pH of the medium was 4.5-5.5 and sterilized by autoclaving (121°C for 15 min). The medium was decanted to remove precipitated protein, dispensed in 250 ml capacity Erlenmeyer flasks (50 ml each) and then re-autoclaved.

2-4. Culture conditions:

Growth was carried out at 28°C under shaking conditions (180 rpm) for 5 days.

2-5. Inoculums:
The inoculums was prepared from 2-3 day old cultures. The organisms from a test tube slope were suspended in 5ml sterile distilled water and then 1 ml suspension was directly inoculated in the Erlenmeyer flasks containing 50 ml medium.
2-6. Biomass yield, Dry cell weight:
The cells were harvested by centrifugation, washed twice with dist. water and the dry weight determined after drying over night (6-8 hours) at 105°C.

3. RESULTS AND DISCUSSION

After preparation of cheese whey, inoculation of yeast strains (Table 1) and incubation in 28°C, the biomass yield of the pour culture of the isolated yeast strains was detected (Table 2).
The yeasts which were able to grow on whey (W) are shown in Table 1. Preliminary tests in our laboratory showed that among the yeasts chosen in this survey, 2 yeast strains (*Cryptococcus laurentii* and *Cryptococcus flavus*, respectively) as shown in the table 2 and 3 had relatively high biomass production when cultivated on cheese whey. These 6 yeast strains were experimented with for growth studies and biomass or bioprotein production when utilizing sterilized, non-sterilized and enrichment cheese whey.
From Table 2, it could be concluded that S1, the non-sterile whey (n-SW), supported more biomass production with all yeasts, but except for (***Cryptococcus podzolicus***). The types of whey used showed significant effect $P<0.001$ on the biomass production of yeasts.

Table 2. Growth of yeasts when utilizing whey in different treatments of substrates (g/lit.Dry cell weight)

No.	Strains	Substrates				
		C I	C II	S1	S2	S3
1	***Cryptococcus laurentii***	18.83	18.72	19.03	18.74	18.89
2	***Cryptococcus flavus***	16.82	16.89	16.81	15.68	17.12
3	***Trichosporon pullulans***	8.88	9.09	13.14	14.18	15.51
4	***Candida curvata***	11.14	11.06	10.59	10.17	12.34
5	***Bullera oryza***	11.33	11.51	12.73	12.01	7.66
6	***Cryptococcus podzolicus***	12.99	13.60	9.50	8.21	9.75

On the other hand, the statistic analysis of results in the yeast strains effect on biomass production was shown in table 3.
In non-sterile cheese whey medium without any supplementation, the ***Cryptococcus laurentii*** and ***Cryptococcus flavus*** strains had the most biomass or single cell protein (SCP) production with biomass of 19.03 and 16.81 g/l dry biomass yield respectively (Table 2). Amount of SCP production can be improved with addition of ammonium sulphate and other minerals as nitrogen supplementation. This is in agreement with results obtained later (Cristiani-Urbina et al, 200; Moeine et al, 2004 and Omar,1991). We found that enrichment had significant different effect on biomass yield (Table 2). The amount of produced biomass of ***Cryptococcus flavus, Trichosporon pullulans*** and ***Candida curvata*** strains increased from 15.68, 14.18 and 10.17g/l in whey without supplementation to 17.12, 15.51 and 12.34 g/l dry biomass yield respectively, in the

present of nitrogen supplementation. In this condition, the ***Cryptococcus laurentii*** and ***Cryptococcus flavus*** strains were found to have the highest biomass yield, 18.89 and 17.12 g/l, respectively.

Table 3. The mean of biomass yield of yeast strains when utilizing whey in different treatments (g/lit. Dry cell weight)

Strains	Mean±m of biomass production g/l (Dry wt)
Cryptococcus laurentii	18.842 ± 0.501[a]
Cryptococcus flavus	16.661 ± 1.310[б]
Trichosporon pullulans	12.159 ± 2.787[c]
Candida curvata	11.052 ± 0.917[d]
Bullera oryza	11.048 ± 1.887[d]
Cryptococcus podzolicus	10.812 ± 2.352[d]

a, b, c,… show significant in ($P<0.001$)

On the other hand, The amount of produced biomass of ***Bullera oryza*** strain decreased from 12.01 g/l in whey without supplementation to 7.66 g/l dry biomass yield respectively, in the present of nitrogen supplementation.
The purpose of using non-sterilized sweet or unsalted whey (**n-SW**) was due to an economical point of view namely to lower the cost of process, especially in our country. This whey (**n-SW**) clearly improved the biomass production of the organism used which might be due to the presence of the natural microflora of milk. Also was reported that traditional industrial fermentations are not run under sterile conditions because of costs. Complex flora are involved; reasons for the balance of phase mixed cultures are not always clear (Omar, 1991).

4. CONCLUSIONS

It also would be of considerable value if an environmentally safe microorganism could utilize the lactose of whey permeate and generate potentially profitable quantities of a commercially useful by-product. However, most microorganisms have limited or no β-galactosidase activity, which prevents them from making effective use of lactose as a carbon source.
Further investigations on the production of single-cell oil (SCO) is being undertaken to make full use of an important waste, cheese whey, in Islamic Republic of Iran and Azerbaijan Republic.

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